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# A comparison of the blood oxygen capacity in painted turtles (*Chrysemys picta* Schneider) and box turtles (*Terrapene carolina* Linne)

Harold J. Payne

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A COMPARISON OF THE BLOOD OXYGEN CAPACITY  
IN PAINTED TURTLES (Chrysemys picta Schneider) AND  
BOX TURTLES (Terrapene carolina Linne)

BY

HAROLD JACKSON PAYNE

A THESIS  
SUBMITTED TO THE GRADUATE SCHOOL OF  
THE UNIVERSITY OF RICHMOND IN PARTIAL  
FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF  
MASTER OF ARTS

AUGUST, 1957

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## INTRODUCTION

Among factors necessary for an animal to maintain life is the presence of an oxygen carrier that will transport a sufficient quantity of oxygen necessary for respiratory metabolism. In animals whose blood contains hemoglobin, which is a pigment that loads and unloads oxygen, this carrying capacity is referred to as blood oxygen capacity. It is a measure of the amount of oxygen that will combine with whole blood when completely mixed with air. It is usually expressed in volumes per cent (ml. O<sub>2</sub>/100 ml. blood) and corrected to standard temperature and pressure.

A search of available literature revealed that there were very few reports of total blood oxygen capacity values in reptiles. The only two reports in turtles were of 8 of the aquatic form Pseudemys concinna Le Conte with an oxygen capacity range of 6.6 to 10.8 volumes per cent (Southworth and Redfield, 1926) and of 3 specimens of Pseudemys troostii Holbrook with a mean oxygen capacity of 6.7 volumes per cent (Wilson, 1939). In a series of investigations of the physiological properties of reptilian blood by Dill and Edwards (1935), the blood from eleven alligators was pooled into five blood samples. The mean oxygen capacity was 6.7 volumes per cent. Hopping (1923) reported a range of 9.5 to 15.3 volumes per cent for six alligators. Dill and Edwards (1931) found an oxygen capacity range of 8 to 10 volumes per cent in three crocodiles. The blood of Gila Monsters was treated by Edwards and Dill (1935) in a similar manner as they treated the alligator blood. The blood from

thirty-one Gila Monsters was pooled into eleven samples, which had a mean of 7.6 volumes per cent.

The present investigation is concerned with the comparison of the blood oxygen capacities of two species of turtles, an aquatic form, Chrysemys picta Schneider and a terrestrial form, Terrapene carolina Linne'. Statistics were applied to the data obtained to ascertain whether there were any significant differences between the two species and also to determine if sexual dimorphism exists.

## METHODS AND MATERIALS

Eighty-eight turtles were collected in Henrico, Hanover, Chesterfield, and Northumberland Counties between September 19, 1953, and June 6, 1957. Forty specimens represent the aquatic type, Chrysemys picta Schneider, and forty-eight specimens represent the terrestrial type, Terrapene carolina Linne. According to Carr (1952) Chrysemys picta inhabits ponds, protected lake shores, ditches, slow streams and marshes in North America from New Brunswick southward through the coastal states to Northern Florida; westward to western British Columbia; southwestward to Texas and New Mexico. Terrapene carolina is found in woods, fields, and marshlands from central Maine southward to the Florida Keys; westward below the international boundary across central Michigan and Wisconsin; from here southwestward to include Illinois, Missouri, eastern Oklahoma and Texas.

The aquatic animals were collected in traps from ponds and lakes throughout the area. Thirty-two specimens were taken from Westhampton Lake on the University of Richmond campus. All terrestrial forms were picked up by hand in woods, fields, and along roadsides. During late fall, winter, and early spring, they were often found beneath decaying logs and fallen leaves.

Types of traps used included baited hooks, wire baskets partially submerged and attached to sunning stations, net rigs (Carr, 1952, Crenshaw, 1953) and a floating trap designed and constructed by the author. This trap was constructed as follows (Fig. 1 and 2): A



KEY TO FIGURES 1 AND 2

a = Board or log frame

b = Wire floor

c = Bait (salted fish)

d = Ramp or vaulted side

e = Animal about to enter trap

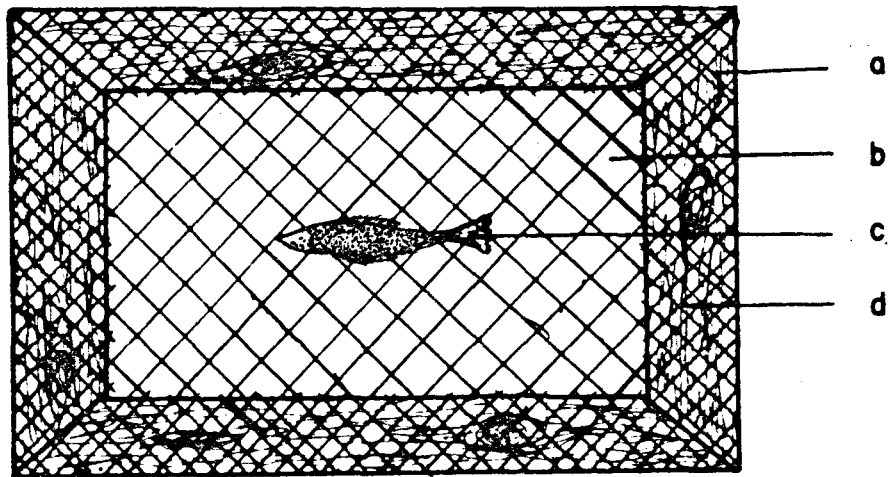


FIG. 1 - TOP VIEW



FIG. 2 - SIDE VIEW

rectangular frame of boards or logs was covered with hardware cloth and/or chicken wire. This formed a floor and served as a bouyant base. Hardware cloth was then nailed on each side of the frame and bent inwardly so that each side formed a ramp. The top center of the trap was left open to serve as the entrance. The inside of the trap resembled a chamber with vaulted sides and an open ceiling. Salted fish or chicken viscera were placed on the floor as bait. In attempting to reach the bait, the animals crawled up the ramp and fell through the opening, and thus were impounded by the vaulted sides. The floating trap yielded an average of one animal per collection while the other types averaged about one animal for every five collections. Because of its obvious superiority, full attention was given to the construction and employment of the floating trap, and all other types were abandoned. The sluggish movements of the box turtles eliminated the necessity of any capture devices other than the collector's hand.

Captured specimens were taken to the laboratory and their weight to the nearest 0.1 of a gm., carapace length and width in mm., and height in mm. (measurement taken from highest central lamina of the carapace to the ventral surface of the plastron) were recorded with the date habitat and location of collection. Rectal temperatures in °C were recorded for each specimen just before an oxygen capacity determination was made.

The blood oxygen capacities of all turtles were determined by the microgasometric syringe method as described by Roughton-Scholander (1943) and modified by Grant (1947). This method was particularly adaptable to the present investigation because the oxygen capacities of small as

well as large animals could be obtained, as only  $39.3 \text{ mm}^3$  of blood are required for a determination.

The Grant modification of the Roughton-Scholander Syringe consisted of a 1 cc. Pyrex tuberculin syringe with an arresting clip on the plunger to prevent slipping and a Pyrex capillary with a 0.5 mm. precision standard bore fused to the syringe nozzle. The capillary top was expanded into a cylindrical cup of 2.5 mm. bore and 1.5 cm. length. The capillary was graduated into fifty divisions, each of which was 2 mm. in length. The transfer pipette was made of glass tubing and ground smooth so as to fit into the glass cup of the capillary. Its volume from the tip to an etched mark was  $39.3 \text{ mm}^3$ , the volume of blood required for a determination. The principle of this syringe method involves the extraction of the total gases from the blood and reagents with a potassium ferricyanide solution. The potassium ferricyanide serves to convert hemoglobin to methemoglobin thus rendering the iron of the hemoglobin molecule incapable of further combination with oxygen. This solution contains saponin which lyses the red blood cells, and also potassium bicarbonate which furnishes excess carbon dioxide that serves as an extraction or carrying medium of the blood and reagent gases. Caprylic alcohol is added to prevent formation of transverse blood films in the capillary of the syringe. A sodium acetate buffer is used in the procedure to stabilize the pH of the blood system. Forty-five per cent urea is added as a cleaning agent because of its protein dissolving property and serves to clean the sides of the capillary and syringe. The carbon dioxide is absorbed by ten per cent sodium hydroxide. A residual gas bubble is driven into the capillary

where its volume ( $V_1$ ) is measured. The oxygen of the bubble is absorbed by addition of alkaline pyrogallol, and the remaining bubble is driven into the capillary and its volume measured ( $V_2$ ). A pre-determined blank value for the dissolved oxygen in the reagents (c) was subtracted from the last measurement and the remainder multiplied by the appropriate correction factor (f) for temperature and barometric pressure, as determined from a nomogram (Peters and Van Slyke, 1932). Thus, the formula for oxygen capacity calculation is  $(V_1 - V_2 - c) \times f = \text{blood oxygen capacity in volumes per cent}$  (Roughton-Scholander, 1943, Grant, 1947).

Each animal was washed and rinsed in distilled water in preparation for obtaining a sample of its blood. The specimen was then decapitated with a clean pair of scissors and held in a position so that the blood dripped into a well of a paraffin coated test plate. The blood was drawn immediately into a heparinized transfer pipette, placed in the syringe, and an analysis made. Two analyses were made on each of eight specimens in order to check sampling and analysis procedure.

The blood oxygen capacity data were recorded along with the previously recorded field data on combination Laboratory - Field Data Sheets (page 8).

The statistical methods of Arkin and Colton (1950) and Simpson and Roe (1939) were used.

LABORATORY - FIELD DATA SHEET

No. \_\_\_\_\_ Sex \_\_\_\_\_ Species \_\_\_\_\_

Date \_\_\_\_\_ State \_\_\_\_\_ County \_\_\_\_\_

Shell. Lg. (mm.) \_\_\_\_\_ Shell Wd. (mm.) \_\_\_\_\_ Dorsal Ventral (mm.) \_\_\_\_\_ Wt. (gm.) \_\_\_\_\_

Remarks \_\_\_\_\_

---

OXYGEN CAPACITY OF BLOOD IN VOLUMES % (ml/100 ml.)

		BLANK					SAMPLE				
Date	Wt. (gm.)	V <sub>1</sub>	V <sub>2</sub>	V <sub>1</sub> -V <sub>2</sub>	c	f	V <sub>1</sub>	V <sub>2</sub>	V <sub>1</sub> -V <sub>2</sub>	c	f

mm. Hg	Room T. °C.	Rectal T. °C.	Volumes%	
			Blank	Sample

Calculations:

$(V_1 - V_2 - c) \times f = \text{Blood oxygen capacity in volumes per cent.}$

## RESULTS

In Tables 1 and 2, the sex, body weight in gm., carapace length, carapace width, and the height in mm., and the blood oxygen capacity in volumes per cent are presented for forty Chrysemys picta and forty-eight Terrapene carolina, respectively. These data are arranged so that the blood oxygen capacities are in ascending order. The blood oxygen capacity range extends from 2.8 to 13.3 volumes per cent in Chrysemys picta and from 2.5 to 11.8 volumes per cent in Terrapene carolina, while the weight range extends from 81.5 gm. to 419.5 gm. for Chrysemys picta and from 37.2 gm. to 471.3 gm. for Terrapene carolina.

Table 3 includes the sex, the number of specimens, the mean body weights in gm., the mean oxygen capacities in volumes per cent, the standard deviations of the mean oxygen capacities and the standard error of the difference between the mean oxygen capacities. Significance tests as well as the mean rectal temperatures for only the total samples were also tabulated. Mean values of the carapace length, carapace width and the height were not recorded because no relationship existed between those measurements and blood oxygen capacity. Table 4 is a compilation from the literature and the present investigation of the mean blood oxygen capacities in volumes per cent for various turtles. The values obtained for Clemmys guttata Schneider and Chelydra serpentina Linne', incidentally determined, were 6.0 and 9.7 volumes per cent, respectively.

In checking, sampling and analysis procedure, the two analyses made on each of 8 turtles varied no more than 0.2 volumes per cent.

At the time blood was obtained from each animal, it was noticed that it clotted in less than one minute in the well of the paraffin coated test plate. However, when left standing in the specimen, the blood did not clot for at least fifteen minutes.



KEY TO ABBREVIATIONS

TABLE 1.

Wt. In gm.	=	Weight in grams
C.lg./C.wd. In mm.	=	Carapace length over carapace width in millimeters
D-V In mm.	=	Greatest dorsal to ventral measurement in millimeters
Rectal Temp. °/C	=	Rectal temperature in degrees centigrade
B. O. C. (Vols.%)	=	Blood oxygen capacity in volumes per cent

TABLE 1.

Chrysemys picta

Sex	Wt. In. gm.	C.lg./C.wd. In mm.	D-V In mm.	Rectal Temp. °/C	B.O.C (Vols.%)
M	81.5	84/68	31	26.5	2.8
M	117.8	99/76	35	27.5	3.4
M	173.8	108/87	39	25.0	3.5
F	123.5	99/74	35	22.0	4.4
F	310.5	131/95	55	23.3	4.6
F	348.9	133/99	51	22.8	4.6
F	75.5	80/66	31	27.8	4.7
F	62.9	74/64	23	24.7	4.8
F	388.8	148/108	55	22.0	4.8
M	141.5	113/84	39	24.0	4.9
F	231.4	120/86	44	23.5	5.4
M	209.1	108/80	45	22.0	5.6
F	207.0	111/87	46	25.0	5.6
M	197.0	113/88	36	24.0	5.6
F	85.5	87/71	33	27.0	6.0
M	216.3	125/91	39	21.5	6.2
F	310.8	138/101	42	20.0	6.2
M	201.3	118/92	38	25.8	6.3
F	205.8	120/93	39	26.0	6.4
F	353.6	135/101	53	22.0	6.6
F	193.5	108/91	52	23.5	6.9
F	143.9	101/78	38	26.0	6.9
F	221.6	116/84	46	24.9	7.0
F	247.5	127/97	48	19.9	7.0
F	318.5	134/97	57	23.0	7.0
F	222.8	115/86	42	21.5	7.2
F	350.9	130/98	55	23.3	7.4
M	234.3	119/85	50	23.5	7.4
M	223.5	126/93	41	26.2	7.7
M	168.2	111/79	38	24.3	7.8
M	220.7	116/89	46	20.1	7.9
M	82.3	82/68	32	24.3	7.9
M	125.7	101/79	36	25.4	8.0
M	202.5	117/91	39	23.0	8.4
F	376.0	146/98	52	21.5	8.4
F	240.4	121/92	41	23.0	8.8
F	287.4	126/90	50	25.0	9.0
F	296.3	127/92	53	20.0	9.9
M	175.3	110/89	40	22.0	10.6
F	419.5	153/107	61	22.8	13.3

KEY TO ABBREVIATIONS

TABLE 2.

Wt. In gm.	=	Weight in grams
C.lg./C.wd. In mm.	=	Carapace length over carapace width in millimeters
D-V In mm.	=	Greatest dorsal to ventral measurement in millimeters
Rectal Temp. °/C	=	Rectal temperature in degrees centigrade
B. O. C. (Vols.%)	=	Blood oxygen capacity in volumes per cent

TABLE 2

Terrapene carolina

Sex	Wt. In gm.	C.lg./c.wd. In mm.	D-V In mm.	Rectal Temp. °/C	B.O.C. (Vols.%)
M	355.1	116/100	63	24.5	2.5
M	315.9	118/95	65	24.3	2.8
F	339.6	121/93	67	23.9	3.4
M	297.3	120/99	64	25.0	3.6
F	277.3	123/96	69	24.0	3.6
M	448.4	146/113	65	24.0	3.7
F	281.5	117/93	65	26.8	3.9
F	286.5	137/93	65	25.8	3.9
M	298.2	121/98	64	25.5	4.1
F	331.3	121/95	66	25.0	4.3
M	406.8	143/123	68	26.5	4.5
F	243.0	110/79	56	28.0	4.6
M	244.8	118/97	60	21.9	4.7
M	289.3	117/95	60	22.8	5.0
M	388.6	129/115	64	23.0	5.0
F	391.6	124/100	65	23.2	5.1
M	292.6	119/99	63	24.3	5.1
F	326.6	120/97	68	22.0	5.1
M	160.0	106/84	59	26.0	5.2
F	59.7	75/62	39	23.0	5.2
M	364.9	121/98	67	27.0	5.2
M	382.5	139/122	66	26.5	5.4
F	310.7	117/95	63	24.0	5.4
M	333.9	123/98	68	22.0	5.8
F	205.7	110/97	54	17.8	5.9
M	327.8	124/86	67	26.0	5.9
F	37.2	63/54	34	26.0	6.1
F	378.5	130/118	67	24.3	6.2
M	328.4	119/96	64	25.0	6.2
M	364.5	121/98	66	22.0	6.3
M	471.3	145/109	68	23.0	6.6
F	372.7	128/100	69	21.7	6.6
F	82.7	98/89	58	25.0	6.8
F	327.2	121/97	67	23.5	6.8
M	360.2	119/94	62	23.6	6.8
M	325.2	137/117	63	18.1	7.1
M	404.8	127/100	71	21.0	7.1
M	181.2	109/90	58	27.2	7.2
F	111.0	107/88	56	28.0	7.2
M	343.1	121/94	67	24.0	7.3
F	267.0	116/95	63	25.6	7.4
F	140.4	112/86	56	25.6	7.4
M	399.2	143/120	67	22.8	8.2
F	294.5	115/94	64	22.7	8.7
F	344.1	124/102	75	26.05	8.7
M	276.7	117/90	64	27.0	8.9
F	421.1	146/124	69	26.0	9.1
F	363.9	114/95	67	23.0	11.8

TABLE 3.

Statistical Analysis of Data Showing Blood Oxygen Capacity Relationships Between Chrysemys picta And Terrapene carolina And Between Sexes Within Each Species

M A L E						F E M A L E					(A.D.)	(3 x S.E. <sub>d</sub> )
	No.	B.W.m In gm.	B.O.C.m (Vols.%)	S.D.	S.E. <sub>d</sub>	No.	B.W.m In gm.	B.O.C.m (Vols.%)	S.D.	S.E. <sub>d</sub>		
<u>Chrysemys picta</u>	16	173.1	6.5	2.00	0.64	24	250.9	6.8	2.00	0.64	0.3	1.9
<u>Terrapene carolina</u>	24	334.4	5.4	1.42	0.50	24	273.6	6.3	2.02	0.50	0.9	1.5
TOTAL SAMPLE												
						(A.D.)	(3 x S.E. <sub>d</sub> )	MEAN RECTAL TEMPERATURE (°C.)				
<u>Chrysemys picta</u>	40	219.83	6.6	2.02	0.41	0.7	1.2	23.6				
<u>Terrapene carolina</u>	48	321.21	5.9	1.81	0.41			24.2				

TABLE 4.

Blood Oxygen Capacities in Volumes  
Per Cent From Available Literature  
and the Present Investigation

Family	No.	B.O.C.m (Vols.%)	Method	Authority
Emydidae				
<u>Chrysemys picta</u>	40	6.7	Syringe	Payne
<u>Terrapene carolina</u>	48	5.9	Syringe	Payne
<u>Clemmys guttata</u>	4	6.0	Syringe	Payne
<u>Pseudemys concinna</u>	8	8.1	Van-Slyke modification	Southworth and Redfield (1926)
<u>Pseudemys troostii</u>	3	6.7	Van-Slyke & Neill	Wilson (1939)
Chelydridae				
<u>Chelydra serpentina</u>	1	9.7	Syringe	Payne

## DISCUSSION

It was found that there was neither a significant difference between the mean oxygen capacities of the two species nor between the mean oxygen capacities of males and females within each species. According to Arkin and Colton (1950) the difference between two means is significant and not due to chance alone, only when the difference is greater than three times the standard error of the difference between the means.

Each individual oxygen capacity value falls within its respective statistical range for a given population, except 13.3 volumes per cent in Chrysemys picta and 11.8 volumes per cent in Terrapene carolina. According to Simpson and Roe this range is established when adding or subtracting three standard deviations to or from the mean of the population. Thus, 99.7% of the individuals will be included within three standard deviations from the mean. For Chrysemys picta total sample:  $6.6 \pm (3 \times 2.0)$ ; male =  $6.5 \pm (3 \times 2.0)$ ; female =  $6.8 \pm (3 \times 2.0)$ . For Terrapene carolina total sample:  $5.9 \pm (3 \times 1.8)$ ; male =  $5.4 \pm (3 \times 1.4)$ ; female =  $6.3 \pm (3 \times 2.0)$ .

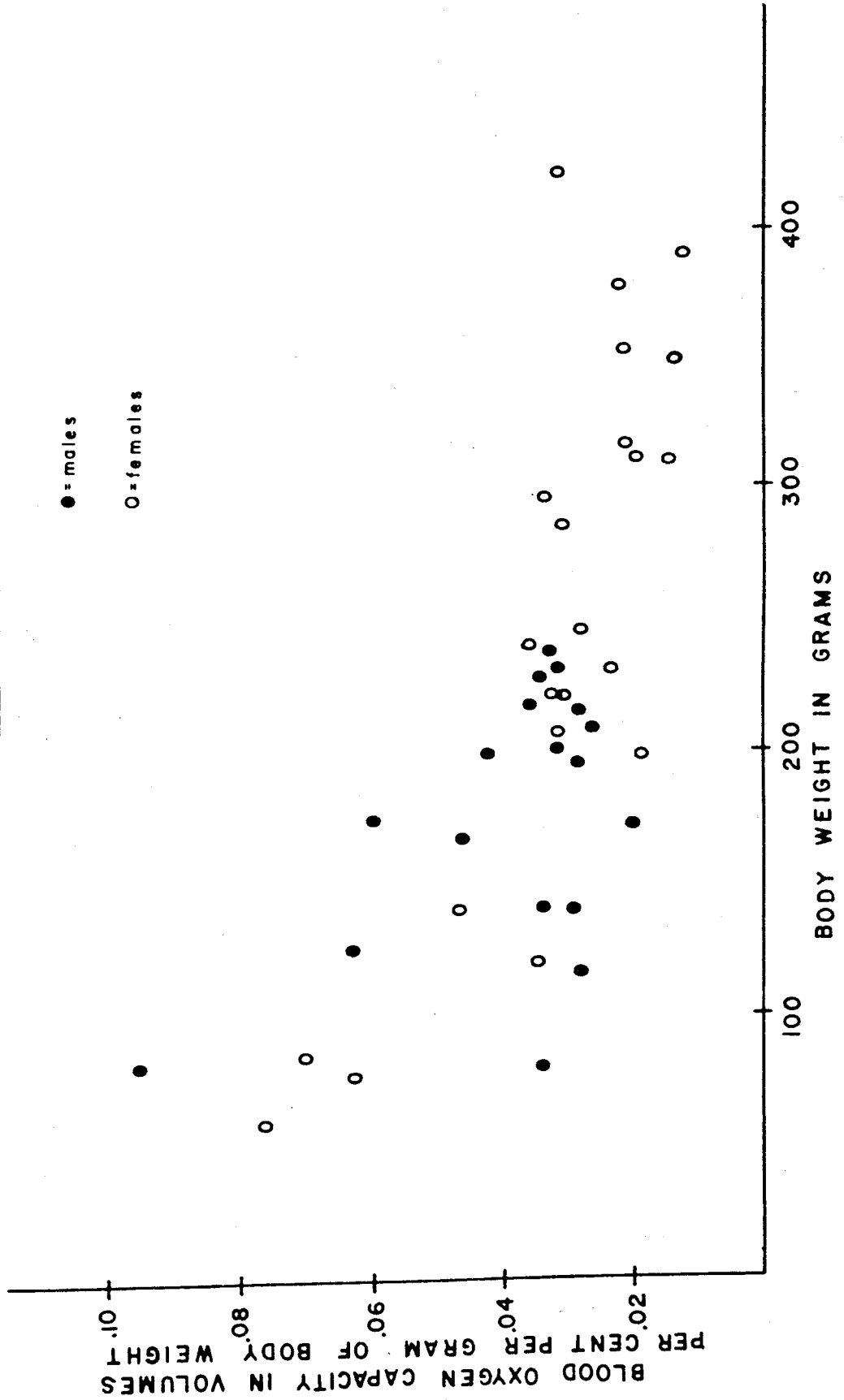
A relationship did exist, however, between body weight and blood oxygen capacity. As the body weight increased there was a tendency for the oxygen capacity to increase. A marked inverse relationship also existed between blood oxygen capacity per gm. of body weight and body weight in gm. as shown in Graphs 1, 2, and 3 where blood oxygen capacity in volumes % per gm. of body weight was plotted against gm. of body weight;

GRAPH 1.

Relationship of blood oxygen  
capacity per gram of body weight  
to body weight in grams in  
Chrysemys picta.



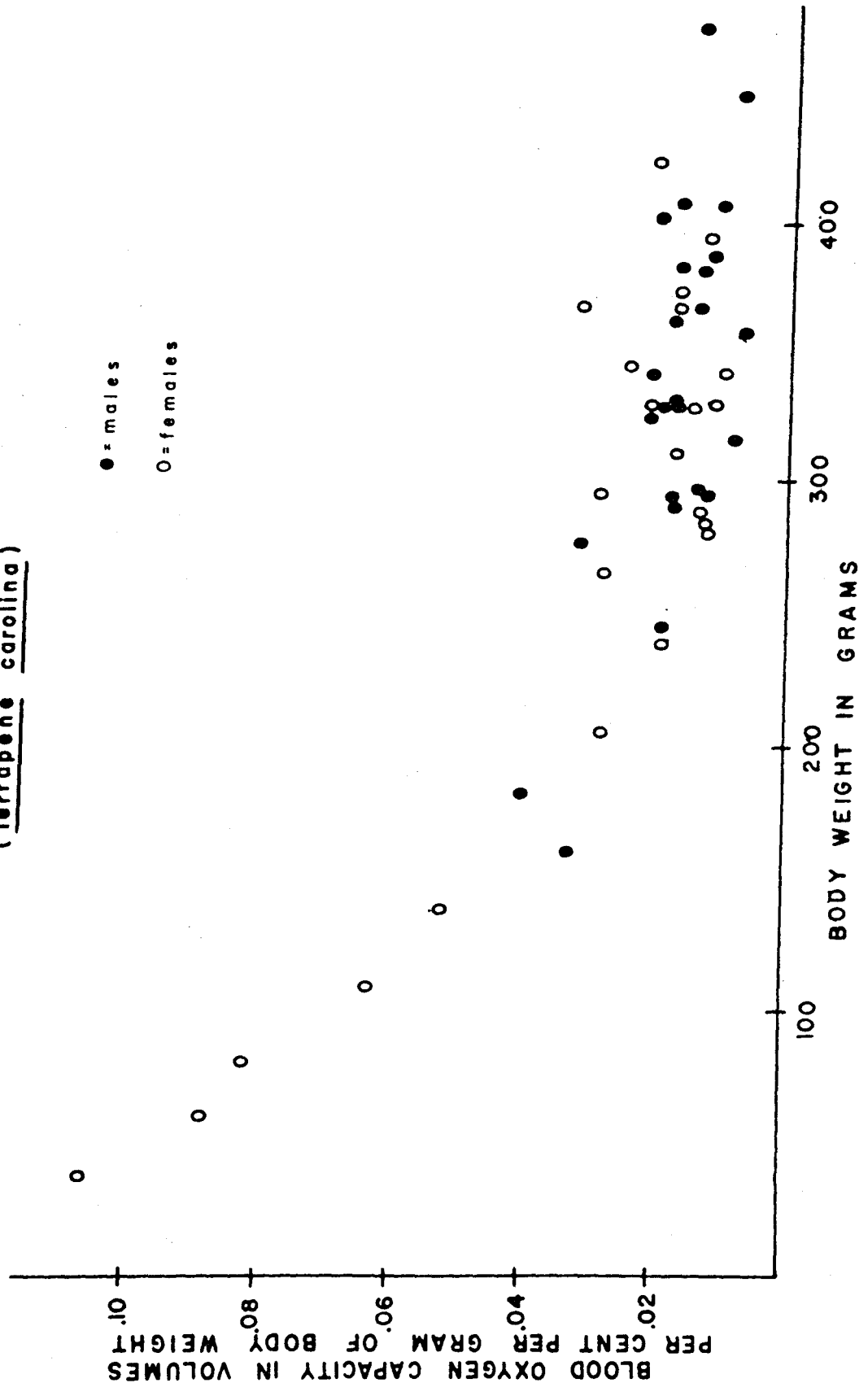
GRAPH I.  
*(Chrysemys picta)*



GRAPH 2.

Relationship of blood oxygen  
capacity per gram of body weight  
to body weight in grams in  
Terrapene carolina.

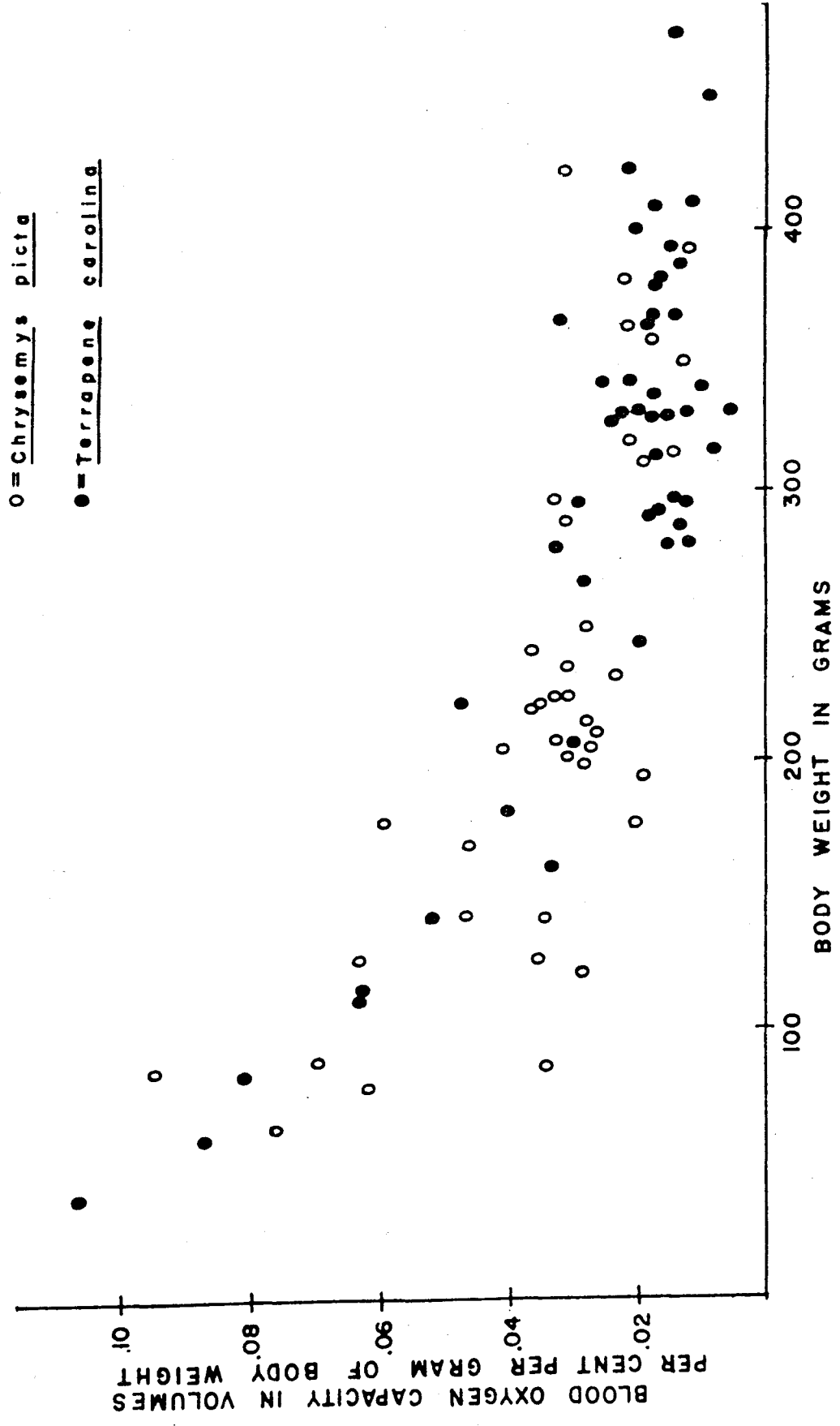
GRAPH 2.  
(Terrapene carolina)



GRAPH 3.

Relationship of blood oxygen  
capacity per gram of body weight  
to body weight in grams for the  
total sample.

GRAPH 3



as the body weight increased, the blood oxygen capacity per gm. of body weight decreased. Graph 1 represents this relationship for forty aquatic turtles while Graph 2 represents the same relationship for forty-eight terrestrial turtles. In Graph 3, the inverse relationship is shown for the total sample of each species. The direct relationship of oxygen capacity to body weight and the inverse relationship of oxygen capacity per gm. of body weight to body weight in gm. was found to exist in mammals by Burke (1957) and in fish by Burke and Woolcott (1957) but has not been shown in other vertebrate classes. The same relationships in turtles, as shown here, have not been demonstrated prior to the present investigation.

Prosser, et al. (1952) discuss oxygen affinity for blood in terms of oxygen tension or pressure measured at the one-half saturation level ( $T \frac{1}{2}$  sat) in millimeters of mercury. In a series of seven turtles, Terrapene carolina was reported to have the lowest oxygen pressure or highest affinity for the blood ( $T \frac{1}{2}$  sat = 12.0 mm. Hg), whereas aquatic turtles had lower oxygen affinities, the pressure when one-half saturated for Chrysemys picta being 15.0 mm. and for a marine turtle, Caretta caretta, 28.5 mm. (McCutchen, 1957). This condition of similar blood oxygen capacities with a great difference in oxygen affinity values exists in catfish (Black, 1940) and trout (Irving, Black and Safford, 1941). Further investigation should be made into the blood properties of Chrysemys picta and Terrapene carolina, and the relationships between the species compared. McCutcheon has shown that the oxygen affinity of the box turtle, being higher than that of aquatic turtles, is on first

consideration at variance with comparisons made in other vertebrate classes. He explains, "Whether embryo of reptile, bird or mammal is compared to adult; tadpole to frog; or aquatic amphibian to terrestrial amphibian, aquatic habitat is consistently correlated to Hb with a higher loading but lower unloading capacity for oxygen, however, these are not valid analogies, since turtles are primarily air breathing even in aquatic habitat. Marine turtles, with the shell no longer an encumbrance, are very active in contrast to the sluggish terrestrial turtles. The correlation of behavior in aquatic turtles with a type of Hb which has a high unloading capacity for oxygen as compared with terrestrial types seems apparent. A further factor may be the diving habits of aquatic species."

## SUMMARY

1. Forty specimens of the aquatic species (Chrysemys picta) and forty-eight specimens of the terrestrial species (Terrapene carolina) were collected for subsequent blood oxygen capacity determination.
2. An original type of floating trap was designed and constructed by the author for capture of the aquatic species. It was unique in that its sides served as an approach ramp to the entrance of the trap. Terrestrial species were picked up by hand.
3. All blood oxygen capacities determined were by the Grant modification of the Roughton-Scholander microgasometric syringe method.
4. Blood from each animal coagulated in less than 1 minute in a well of a paraffin coated test plate; however, left standing in the specimen, it did not clot for at least fifteen minutes.
5. Statistically, there was neither a significant difference between the mean oxygen capacities of the two species nor between males and females within each species. The mean values in volumes per cent were: Chrysemys picta total sample = 6.6, male = 6.5, female = 6.8; Terrapene carolina total sample = 5.9, male = 5.4, female = 6.3.
6. As the body weight increased, there was a tendency for the oxygen capacity to increase.
7. As the body weight increased, there was a definite decrease in blood oxygen capacity per gm. of body weight.



8. The oxygen capacities of two other aquatic forms, four Clemmys guttata and one Chelydra serpentina were determined. The mean value for Clemmys guttata was 6.0 volumes per cent, the value for Chelydra serpentina was 9.7 volumes per cent.

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